Secondary Structures of Wheat α- and ω-Gliadin Proteins: Fourier Transform Infrared Spectroscopy

Attempts have been made to interpret Fourier transform infrared (FT-IR) spectra in the amide I region of an α-gliadin protein fraction (A-gliadin) from wheat endosperm in terms of secondary structure and conformation. Spectral resolution enhancement techniques (Fourier self-deconvolution) proved to be of limited value in analyzing the FT-IR spectra to determine the frequencies of the broad amide I band, perhaps because amide I component absorption bands were found to be broader and more overlapping in comparison with those characteristic of many proteins and enzymes of known primary and secondary structure. Glutamine makes up 34 mole per cent of the amino acids of A-gliadin, and results were corrected for absorptions of primary amide groups of glutamine side chains that contributed strongly to the spectra near 1635 cm^{-1}. Corrected FT-IR spectra of an ω-gliadin protein likely to have large numbers of beta-turns and of cyanogen bromide-cleaved peptides of A-gliadin were also obtained, and provided support for a potential amide I component due to beta-turns near 1658 cm^{-1}. Results from FT-IR, circular dichroism (CD) and predictions from the primary structure of A-gliadin were combined to suggest that the A-gliadin structure has about equal proportions of the four structural components, α-helix, linear β-structure, β-turns and unordered structure (within ± 10 %).

Introduction

The various conformational structures of the wheat storage (gluten) protein components are considered important in determining their contribution to the viscoelastic properties and other quality characteristics of wheat flour doughs and, possibly, to varietal differences in these properties[1, 2]. All storage protein components of wheat have large amounts of glutamine (30–55 mole per cent) and proline (12–32 mole per cent) as a consequence of primary structures made up almost entirely of repeating sequences that include glutamine and proline (ω-gliadins, high molecular weight glutenin subunits) or primary structures that include substantial numbers of such repeating sequences (α-type and γ-type gliadins)[1]. For example, the sequence -Pro-Gln-Gln-Pro-Tyr- occurs frequently in ω-gliadins and has been noted in α-gliadins[1].

There has been relatively little study of the conformational structure of purified protein components from the complex mixture of storage proteins characteristic of

wheat endosperm, however, even though there are considerable differences in composition and primary structure among some components of the mixture[1–3]. Structural information about purified fractions has come mainly from circular dichroism (CD) spectra[4–6]. Recent viscometric[7] and nuclear magnetic resonance studies of the barley C-hordein fraction[8], which is apparently closely related in primary structure to the ω-gliadins of wheat[9], have provided important new information on the presence of β-turns in the conformation of these proteins that is likely to apply to ω-gliadins as well.

Recent applications of resolution enhancement techniques to FT-IR spectra of the amide I region of proteins[10–12] encouraged us to apply these methods to spectra of A-gliadin, a purified α-gliadin protein of known amino acid sequence[13]. We also obtained CD spectra of A-gliadin and predicted its secondary structure, based on these spectra[14] and based on amino acid structure[15]. These results were compared with the results of the FT-IR study. In addition, we compared the FT-IR spectra of A-gliadin with the spectra of two major peptides, CN-I and CN-II, obtained by cleavage of A-gliadin with cyanogen bromide[13]. CN-I corresponds to the first 127 residues and CN-II to residues 128–246 of the 266 amino acid residue polypeptide chain. Finally, we obtained the FT-IR spectrum of a purified ω-gliadin component that is likely to have a structure rich in beta-turns as a possible model for the repeated sequence region (domain I)[13] of A-gliadin, which also makes up a major part of peptide CN-I.

Materials and Methods

Proteins and peptides

A-gliadin was isolated according to the procedure of Bernardin *et al.*[16] Cyanogen bromide-cleaved peptides of A-gliadin were obtained as previously described[13], as was the ω-gliadin preparation[17], which corresponds to the ω-1 component of the cultivar 'Chinese Spring'[17].

Chemicals

Deuterium oxide (99·8 atom per cent) was purchased from Aldrich Chemical Co. Ethanol-d_1 was obtained from MSD Isotopes. All other reagents were of analytical grade.

Spectroscopy

A-gliadin solutions for FT-IR spectroscopy were prepared by adding $10 \pm 0{\cdot}7$ mg of protein to 1 ml of solvent. Deuterated solvents were used because water absorbs strongly in the amide I region around 1640 cm^{-1}. After dissolution in deuterium oxide, 0·005 M KCl or 70% (v/v) ethanol-d_1/deuterium oxide, A-gliadin solutions were allowed to stand for a minimum of 24 h to assure complete H,D exchange. ω-Gliadin and CN peptides were available in limited quantities, and solutions were prepared by dissolving 3 mg or less of protein or polypeptide in 0·1–0·3 ml of solvent and allowed to stand. Deuterium ion concentration, expressed as pD, was adjusted with 1 M DCl to pD 3 or pD 5 as desired. These two pHs were chosen because they had been used in previous studies of A-gliadin[4, 18, 19].

FT-IR spectra were obtained at room temperature with a Nicolet 7199 spectrometer according to previously described procedures[12]. Instrument resolution was 2 cm^{-1} for all spectra. Sample solutions of A-gliadin and solvents were contained in a 150 μm pathlength cell with CaF_2

windows. Solvent-subtracted spectra of protein solutions were required to observe the spectra of the protein. Spectra of peptide and ω-gliadin solutions were obtained at different concentrations and pathlengths as indicated in Results and Discussion.

Circular dichroism spectra in the ultraviolet range were obtained for A-gliadin solutions at pH 3 and pH 5 in water (pH adjusted with HCl) at room temperature (approximately 20°) with a Jasco Model J-500 spectropolarimeter. Samples were contained in quartz cuvettes with pathlengths of 0·1 or 1 mm. Spectra were obtained in the range 185–250 nm. Estimates of the proportions of secondary structure were made according to the method of Chang *et al.*[14]

Resolution enhancement

Fourier self-deconvolution was used to enhance the resolution of the FT-IR spectra as previously described[10–12]. The two deconvolution parameters, half bandwidth at half-height (s) and the resolution enhancement factor (k = ratio of bandwidths before and after deconvolution)[20], were set at 6·5 or 9 cm^{-1} and 2·4 or 2·8, respectively, for this study.

Prediction of secondary structure from primary structure

The Chou-Fasman method[15] was used to predict secondary structure from the amino acid sequence of A-gliadin.

Results and Discussion

A-gliadin spectra

The spectrum of A-gliadin at pD 5 in the range 1550–1750 cm^{-1}, which includes the amide I region is shown in Fig. 1(a) (prior to deconvolution). Spectra obtained at pH 3 and pH 5 in the presence (not shown) or absence of 0·005 M KCl were very similar to this spectrum, although there were slight differences, particularly near 1580 cm^{-1} and 1710 cm^{-1}, between spectra obtained at the different pHs. These latter differences probably result from changes in the ionization of side chain carboxyl groups[21]. The maximum absorption was at 1643 cm^{-1} in all cases.

These results indicate no major changes in A-gliadin conformation under the conditions we studied, which is in agreement with our results from CD spectra and those of a previous CD study[4]. Although no major changes in conformation occur with pH, CD in the near ultraviolet[4] indicates a loss in optical activity of tyrosine and tryptophan side chains and a possible slight decrease in α-helix content at pH 3 in comparison with pH 5. There is a significant increase in viscosity at pH 3 as compared with pH 5[18]. The increase in viscosity at pH 3 might result from a dissociation of domains that retain secondary structure while connected by a short length of polypeptide chain; for example, a rod-like N-terminal region might acquire motional freedom relative to a more globular C-terminal domain. Spectroscopic methods sensitive to changes in secondary structure would not be strongly affected by such a dissociation. All three disulfide bonds of A-gliadin reside in the C-terminal half of the molecule and are likely to stabilize this part of the structure; the N-terminal repeat region and this stabilized C-terminal region might act like independent domains to some extent.

A-gliadin associates to form fibrillar aggregates when the pH is changed from pH 3 to pH 5 in the presence of 0·005 M KCl[19]. This association causes no major change in the

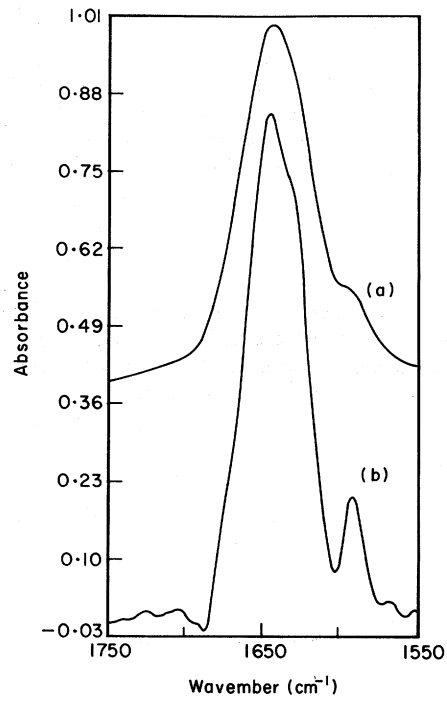

FIGURE 1. FT-IR spectra of A-gliadin (12 mg/ml) in D_2O, pD 5, pathlength 150 μm. (a) Original spectrum offset + 0·39 absorbance units. (b) Deconvoluted spectrum, s = 9, $k = 2{\cdot}4$.

amide I spectrum supporting earlier conclusions that association was not accompanied by anything more than minor conformational changes in the protein molecules[4, 19].

In aqueous ethanol (70% ethanol) at both pD 3 and pD 5 the FT-IR spectrum of A-gliadin differed only slightly (results not shown) from the spectrum of a D_2O solution of A-gliadin. The only noticeable change was a band shift from 1629 to 1632 cm^{-1} for the lowest frequency among the amide I component bands. The presence of 70% ethanol-d_1 at pD 3 apparently increased the ionization of side chain carboxyl groups in A-gliadin in that the weak band at 1587 cm^{-1} in the absence of ethanol due to carboxylate groups appeared as a stronger band at 1595 cm^{-1}. This spectral change probably resulted from an increase in pH, which was not compensated for after initial solution of the protein. Because side chain carboxyl groups titrate mainly in the range pH 3–5, an increase in pH would significantly affect the degree of ionization of these groups.

Comparing the spectra of A-gliadin in 70% ethanol solutions with those we obtained in purely aqueous solutions suggested that the secondary structure is somewhat more clearly defined in the 70% ethanol solution as indicated by slightly more pronounced shoulders in deconvoluted spectra. The spectra in 70% ethanol-d_1 were not characteristic of any major unfolding of the protein[12] and bands near 1615 and 1685 cm^{-1} that had been noted for some other proteins in the presence of alcohol[12] were absent.

Deconvoluted FT-IR spectra

Fourier self-deconvolution of the spectrum in Fig. 1(a) yielded the spectrum in Fig. 1(b). Deconvolution of spectra for other A-gliadin solutions yielded similar results. Neither deconvolution nor second derivative spectra resolved a glutamine side chain amide band from gliadin amide I components nor resolved amide I components significantly. The resolution of the FT-IR spectra of gliadin solutions was only slightly enhanced by deconvolution as indicated by the appearance of shoulders on the broad amide I band. Thus, it proved more difficult to resolve band positions of amide I components than has been the case for other proteins[10, 12, 20]. Although we have considered the deconvoluted spectra to a limited extent in interpreting our spectra, we will not discuss them in detail.

The apparent lack of resolution enhancement in the deconvoluted spectra may result from the presence of a large number of closely-spaced unresolved components in the amide I spectrum of A-gliadin. A similar lack of resolution has been observed in the deconvoluted amide I spectrum of casein micelles[10]. The absence of a discernible pattern of component bands characteristic of particular secondary structures might result from such unresolved components[10]. Components of greater bandwidth, but with similar frequencies to those normal globular proteins would be more difficult to resolve.

Contributions of amide side chains

Gliadins and other wheat storage proteins are noted for exceptionally large proportions of glutamine in their compositions; A-gliadin has 91 residues of glutamine out of 266 residues (34 mole per cent) whereas ω-1 gliadin of Chinese spring is 43 mole per cent glutamine. These amide side chains are both good hydrogen bond donors and acceptors and because of their numbers and distribution in the primary structure must occupy both interior and surface positions of the proteins. Hydrogen bonding of these primary amide side chains to main chain amides might produce distortion of secondary structures, thus contributing to the observed band broadening in the FT-IR spectra.

In addition, Chirgadze *et al.*[21] found that the amide side chains of glutamine and asparagine contribute to absorption in the amide I region of the IR spectra of proteins and polypeptides. We have used their data[21] to correct our FT-IR spectra for the contribution of glutamine side chains (there are only seven asparagine residues in A-gliadin and their contribution was ignored). The contribution of glutamine side chains to a spectrum was calculated from the equivalent molarity of glutamine in our solution (as defined by protein concentration and moles of glutamine in the protein) and the data in Table VI of Chirgadze *et al.*[21], which gives molar absorption coefficients of glutamine as a function of wavelength. Our corrected spectra are the difference between the original spectrum and the glutaminyl side chain spectrum.

Resulting corrected spectra for A-gliadin at pD 3 and pD 5 are shown in Fig. 2. The correction shifts the maximum absorption to 1654 cm^{-1} (pD 3) and to 1651 cm^{-1} (pD 5) from 1643 cm^{-1} in the uncorrected spectra. A shoulder is evident at about 1615 cm^{-1} and weak absorptions were likely near 1675 cm^{-1} based on deconvoluted spectra (the maximum correction for glutaminyl side chains occurs near 1635 cm^{-1} so that correction

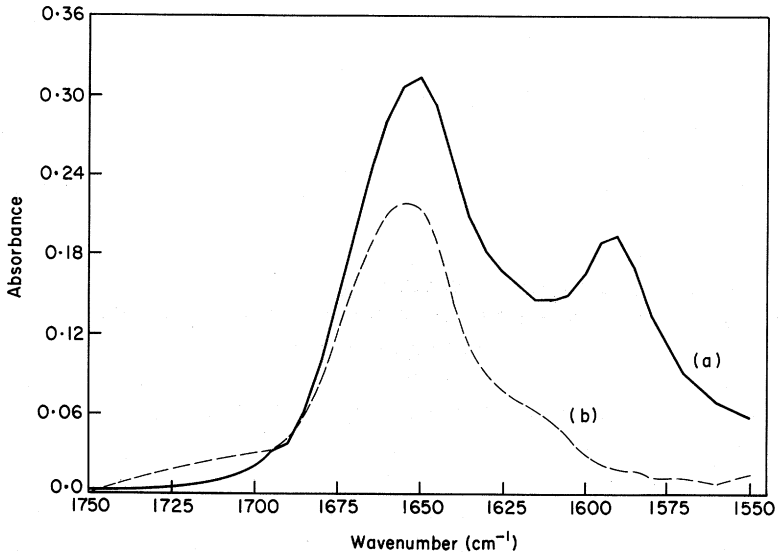

FIGURE 2. FT-IR spectra of A-gliadin after subtraction of glutamine side chain spectrum. (a) A-gliadin 12 mg/ml in D_2O at pD 5, pathlength 150 μm. (b) A-gliadin 12·8 mg/ml in D_2O at pD 3, pathlength 150 μm.

near 1675 cm^{-1} is minimal and results in the latter region may be based on uncorrected spectra.)

Correction for amide side chains leads to the conclusion that the α-helical structure, which absorbs near 1654 cm^{-1} [10], may make a significant contribution to the secondary structure of A-gliadin. The broadness of the absorption band certainly allows for some contributions from random structure with an absorption near 1643 cm^{-1} and β-structure with absorptions near 1630 cm^{-1} and 1675 cm^{-1} [10]. As we shall discuss in a following section, however, β-turns may have an absorption near 1658 cm^{-1} that would overlap with the α-helix absorption near 1654 cm^{-1} and the β-structure absorption near 1675 cm^{-1} in the A-gliadin spectrum.

ω-Gliadin spectrum

We chose to compare the spectrum of ω-gliadin to that of A-gliadin, because the ω-gliadin might serve as a model for the repeated sequence regions in the N-terminal half of A-gliadin[13]. The A-gliadin repeats consist of sequences similar to -Pro-Gln-Pro-Gln-Pro-Phe-Pro-Pro-Gln-Gln-Pro-Tyr- repeated about eight times[13]. Although there is limited sequence data available for our ω-gliadin component, the sequences available, along with compositional data[17], suggest that it is likely to consist almost entirely of repeats similar to, but probably not identical to, the A-gliadin repeats. The composition of our ω-gliadin includes 43 mole per cent glutamine, 29 mole per cent proline and 9 mole per cent phenylalanine, which suggest that the repeats are similar to the octapeptide repeat -Pro-Gln-Gln-Pro-Phe-Pro-Gln-Gln- that has been suggested for the

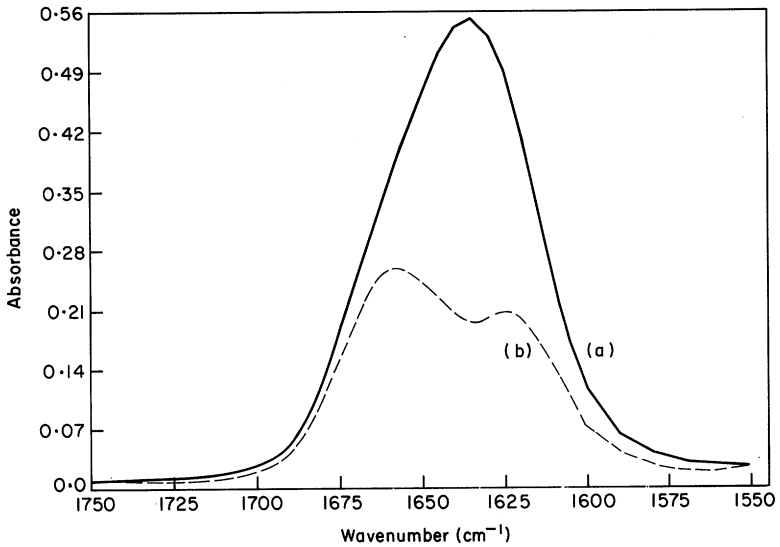

FIGURE 3. FT-IR spectra of ω-1 gliadin. (a) Original spectrum 23 mg/ml, D_2O, pD 3, 75 μm. (b) Spectrum 3(a) after subtraction of glutamine side chain spectrum.

homologous C-hordeins of barley[1,6,22]. Tatham *et al.*[6,8] have provided convincing evidence that the C-hordein structure is rich in β-turns and have shown that ω-gliadins and C-hordeins have similar CD spectra[5,6]. This spectrum with a weak shoulder near 230 nm and a strong negative peak at 203 nm, is likely to be characteristic of β-turns[6].

The FT-IR spectrum of a highly-purified ω-gliadin (ω-1 gliadin from Chinese Spring[17]) uncorrected for amide side chain absorption, is shown in Fig. 3(a). The spectrum exhibited a single broad band with maximum at 1636 cm^{-1}. Correction of the spectrum for 43 mole per cent of glutamine side chains (asparaginyl side chains were considered to make a negligible contribution to the spectrum) yielded the spectrum in Fig. 3(b). The corrected spectrum showed clear evidence of absorption bands near 1623 cm^{-1} and near 1658 cm^{-1} with the likelihood of several bands of intermediate frequencies. Because the ω-gliadin component has, on average, a proline residue at every third position along the polypeptide chain, there is little possibility for α-helix in this protein. It seems reasonable to assign the strong absorption near 1658 cm^{-1} to β-turn structures; secondary structures other than α-helix, which we consider unlikely to exist in ω-gliadins, are unlikely to absorb strongly at this frequency. Absorption in the range 1659 to 1666 cm^{-1} has been assigned to β-turns in the spectra of a series of proteins[10]. The band at 1623 cm^{-1} might result from linear β-structure (β-strands), although the possibility of β-turns having a low frequency component cannot be ruled out.

FT-IR spectra of A-gliadin peptides

Cleavage of A-gliadin with cyanogen bromide yields three peptides. We have examined the FT-IR spectra of the two larger peptides CN-I and CN-II with molecular weights of

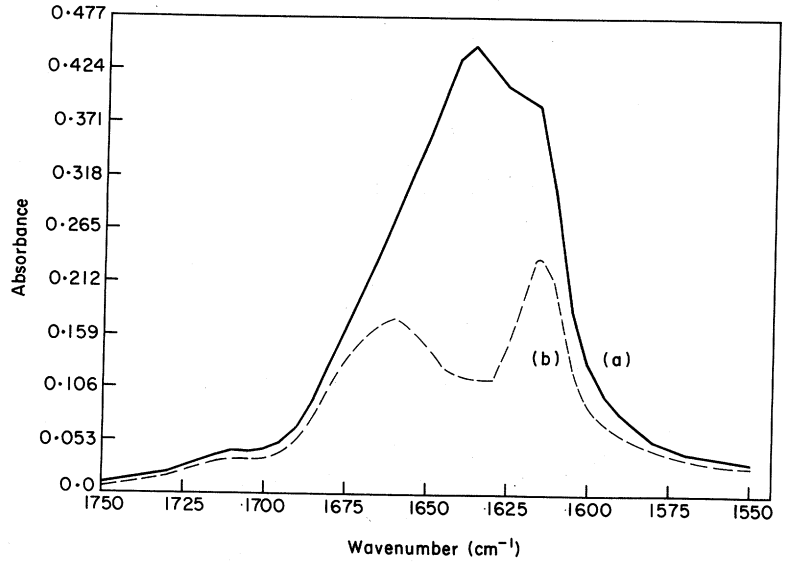

FIGURE 4. FT-IR spectra of CN-I peptide of A-gliadin. (a) Original spectrum 13 mg/ml, D_2O, pD 3, 116 μm. (b) Spectrum 4(a) after subtraction of glutamine side chain spectrum.

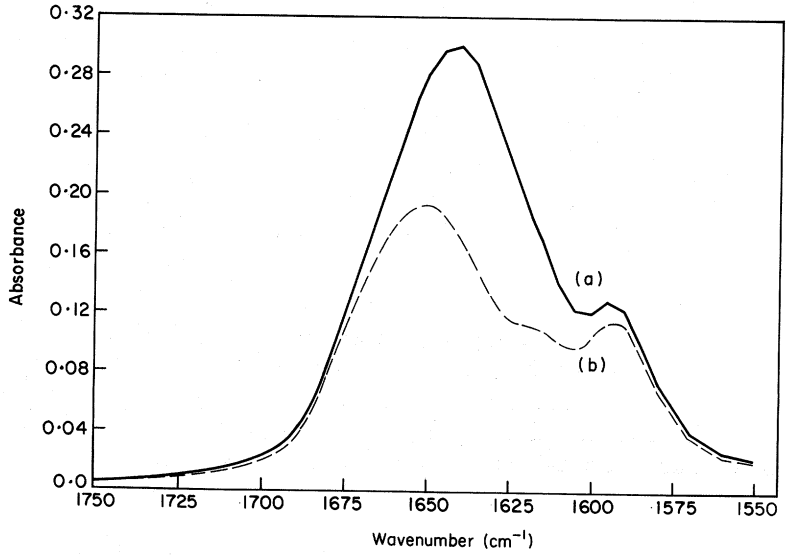

FIGURE 5. FT-IR spectra of CN-II peptide of A-gliadin. (a) Original spectrum 9 mg/ml, D_2O, pD 3, 116 μm. (b) Spectrum 5(a) after subtraction of glutamine side chain spectrum.

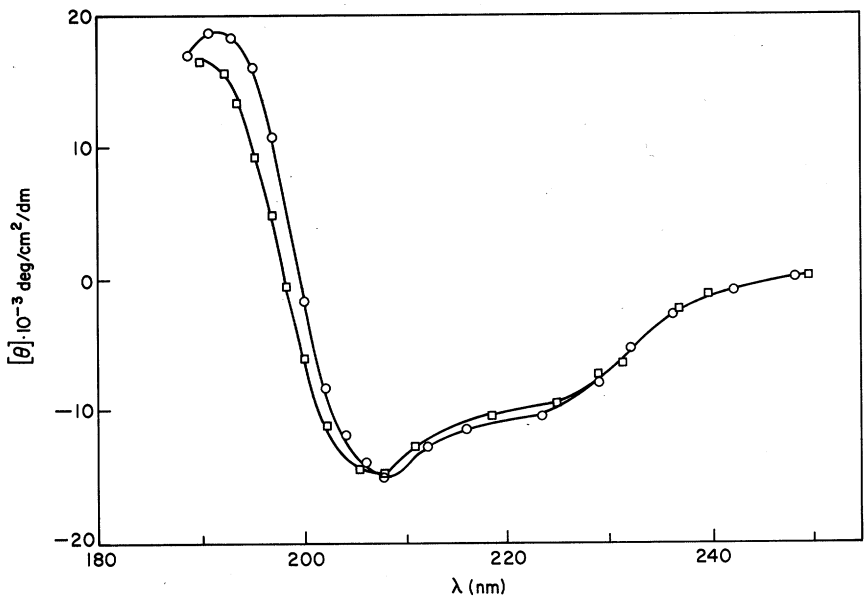

FIGURE 6. Circular dichroism spectra of A-gliadin in water: ○——○, pH 3; □——□, pH 5; pH adjusted with HCl.

15095 and 13655, respectively, that together make up almost 93% of the protein. The C-terminal peptide, CN-III, makes up only about 7·5% of the polypeptide chain and is poorly-soluble under the conditions we used.

Spectra uncorrected for amide side chain absorption are shown in Figs 4(a) (CN-I) and 5(a) (CN-II) and corrected in Figs 4(b) (CN-I) and 5(b) (CN-II). The spectrum of CN-I, which includes the repeating sequences likely to include β-turns, also shows a strong band near 1660 cm^{-1} similar to that found in the ω-gliadin spectrum, thus supporting the likelihood of an important absorption band for β-turns (at least those characteristic of wheat gliadin proteins) near this frequency. CN-I also exhibits a band shape in the amide I region that indicates the presence of other component bands characteristic of β-structures, α-helix or unordered structure. CN-I includes the polyglutamine domain (II) of A-gliadin, which consists of 18 glutamine residues in series[13]. The conformation of this polyglutamine region is not known. The CN-I spectrum also exhibits a strong absorption band at 1615 cm^{-1}. The origin of this band is puzzling in that it did not appear strongly in either of the protein samples. The prominent band at the same frequency paired with a smaller band near 1685 cm^{-1} occurs in the spectra of some globular proteins (not A-gliadin) in aqueous alcohol[12], especially those with a high β-structure content, and may be indicative of a conformation resembling β-structure, but it is not known if there is any connection between these observations[12] and the conformation of CN-1.

The FT-IR spectrum of CN-II (Fig. 5(b)) appeared to have a major absorption near 1652 cm^{-1}, definitely shifted to lower frequency in comparison with the spectrum of

CN-I (Fig. 4(b)). CN-II has much less proline (nine of 119 amino acid residues) than CN-I (58 of 127 residues) and the absorption near 1652 cm^{-1} might result from α-helical structure. A band at 1658 cm^{-1} is not discernible in this spectrum, which might indicate fewer β-turns of the type characteristic of CN-I. Because of the broadness of the bands and the degree of overlap among them, it was not possible to analyze these spectra in detail, but it seems reasonable to suggest the possibility of contributions from random structure (absorptions near 1643 cm^{-1}) and β-structure (absorptions near 1630 cm^{-1} and 1675 cm^{-1}). The absorption band near 1595 cm^{-1} probably results from an increased ionization of carboxyl groups[19]. CN-II includes most of the side-chain carboxyl groups of A-gliadin. The spectral differences between the two peptides of A-gliadin indicate that each folds into a fairly different conformation.

Circular dichroism of A-gliadin

The CD spectrum of A-gliadin in water at pH 3 (pH adjusted with HCl) is shown in Fig. 6(a) and in water at pH 5 in Fig. 6(b). The spectra are in agreement with previously published work[4] within experimental error and extend the results to include the positive band near 190 nm. This spectrum is of the α- + β-type[23] and is rather similar to the CD spectrum of lysozyme[14]. Predictions of secondary structure according to the method of Chang *et al.*[14] indicated that A-gliadin has the following percentages of secondary structures:

pH	α-Helix (%)	β-Structure (%)	β-Turns (%)	Unordered (%)
3	24	33	1	43
5	28	38	0	34

The absence of β-turns is surprising considering the likelihood that this type of secondary structural element makes a significant contribution to the conformation of A-gliadin. The problem seems to lie with the spectrum of β-turns deduced from the structures of proteins that have been analyzed by X-ray crystallography[14]. This derived spectrum bears almost no similarity to the CD spectrum of ω-gliadins or C-hordeins, proteins that seem likely to be rich in β-turns[5, 6, 8, 22], and the method of Chang *et al.*[14] may tend to assign β-turns in A-gliadin to random structure, the spectrum of which resembles the spectrum of β-turns in gliadins (see CD spectra of ref. 5). Turns in the standard proteins used by Chang *et al.*[14] may not be good models for the proline and glutamine rich turns of gliadins.

Prediction of A-gliadin conformation from primary structure

Chou–Fasman analysis[15] of A-gliadin secondary structures based on the primary structure[13] was not completely unambiguous in that some interpretation was required to

sort out regions where conformational potentials were high for more than one type of structure. Analysis favoring β-structure yielded predictions of:

α-Helix (%)	β-Structure (%)	β-Turn (%)	Unordered (%)
5	45	30	20

whereas analysis favoring α-helix yielded predictions of:

α-Helix (%)	β-Structure (%)	β-Turn (%)	Unordered (%)
27	23	29	21

Both approaches yield a prediction of considerable amounts of β-turn structure. It would be difficult to reconcile the CD spectrum, which has a strong negative minimum at 208 nm and significant shoulder near 222 nm with only 5% α-helix, and we favor the interpretation that maximizes α-helix content on the basis of this spectrum.

Conclusions

Our data seem to indicate that β-turns have a moderately strong absorption near 1658 cm^{-1} in the IR (along with weaker bands at other frequencies). Thus, our results may provide support for the conclusion of Tatham and Shewry[5] that gliadins and glutenins include a significant proportion of β-turn structures. Investigation of model peptides based on gliadin sequences would be helpful in defining the IR and CD spectra of the types of β-turns most likely to occur in gliadins.

Resolution enhancement of the amide I region of the FT-IR spectra of gliadins has not been as helpful in extracting component absorptions as it has for many other proteins[10]. Further work may provide the basis for extracting additional information from FT-IR spectra.

The high glutamine content of gliadins necessitates correction of amide I spectra for primary amide absorption near 1635 cm^{-1}. The data of Chirgadze *et al.*[21], based on spectra of amino acid derivatives, was used to correct our protein and peptide spectra, but further investigation of amide side chain absorption in peptide models would help to provide confidence in the quantitative aspects of the data when applied to side chains of proteins.

By combining our data from different methods and allowing for the failure of the CD method to recognize β-turns, we estimate that A-gliadin includes approximately equal proportions (within about $\pm 10\%$) of the various structural components: α-helix, β-structure, β-turns and unordered structure.

The apparent lack of more than minor conformational change in A-gliadin when solution pH is changed from pH 5 to pH 3 in contrast to a doubling of the intrinsic viscosity when this change is made is best explained by postulating acquisition of some motional independence by two domains that retain their secondary structures while remaining connected by a short length of polypeptide chain. The two domains are likely to correspond to an N-terminal domain consisting mainly of the repeating sequence region rich in beta-turns and to a C-terminal domain that is stabilized by the three disulfide bonds of A-gliadin. A-gliadin apparently undergoes no major conformational change in ethanol–D_2O solution as compared with purely aqueous (D_2O) solution.

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